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# Protective effect of aged garlic extract against the oxidative stress induced by cisplatin on blood cells parameters and hepatic antioxidant enzymes in rats

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ABSTRACT

Cisplatin (CP) is one of the most active cytotoxic drugs. However, it has several side effects that are associated with increased oxidative stress. Aged garlic extract (AGE) is a natural product containing different compounds with antioxidant activity. The present study aimed to evaluate the effect of AGE on CP-induced hepatotoxicity. Four equally male rat groups: control, AGE-treated (250 mg/kg once for 21 days), CP-treated (7.5 mg/kg, once intraperitoneal), combined AGE and CP-treated were used. Blood samples were collected to investigate blood picture and serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin (TSB) and albumin. The liver of each rat was excised, cleaned, weighed, rinsed in ice-cold saline and homogenized for assessment malondialdehyde (MDA) level, catalase (CAT) and superoxide dismutase (SOD) activities and level of reduced glutathione (GSH). Histological examination was also carried out. AGE-pretreated rats revealed significant reduction in serum levels of AST, ALT & TSB and increase serum albumin level induced by CP administration. Furthermore, AGE significantly ameliorated CP-induced increase in MDA level and decrease in GSH level, CAT and SOD activities in liver tissue homogenates. Additionally, histopathological and blood picture examinations revealed markedly ameliorated CP-induced toxicity on blood cells parameters and liver structure. Our results prove that AGE has antioxidant and protective effects against CP-induced oxidative stress and changes in parameters of blood cells and liver structure in rats. Thus, it could be used as a dietary supplementation to reduce toxic side effects of anticancer drugs.



## 1. Introduction

Cisplatin (CP), a potent antineoplastic drug, is widely used in the treatment of different solid-organ tumors. However, its clinical use is limited due to its toxic side effects including nephrotoxicity, neurotoxicity, ototoxicity and hepatotoxicity [1]. The toxicity of CP seems to be dose-dependent due to the cumulative effect of the drug [2], where the accumulation of CP produces obvious necrotic changes within the tissues of the affected organs. The generation of reactive oxygen species (ROS) and nitrogen species (NS) is one of the possible mechanisms responsible for CP toxicity through their oxidative stress injury and suppression of the antioxidant defense system [3]. To ameliorate the toxic effect of CP without inhibiting its anti-tumor effects, different experimental studies were carried

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out using a combination of CP with various radicular scavengers, enzyme inhibitors, sulfur-containing antioxidants and natural foods with antioxidant properties [1,4,5].

Natural and herbal products have been used in traditional medicine to treat a variety of diseases including malignancies [6]. The anticancer activities of the extract from a number of herbal plants have been demonstrated. A number of previous studies concluded that herbal medicine might have anticancer effect by enhancing the immune system, including cell differentiation, inhibiting telomerase activities and inducing apoptosis of cancer cells [7].

Garlic (*Allium sativum*) is a worldwide traditional food and dietary supplement. Nowadays, many garlic preparations are used in the medical field including fresh garlic extract, garlic oil, aged garlic extract (AGE) and a number of organosulfur compounds. AGE is an odorless garlic preparation produced by prolonged extraction of fresh garlic at room temperature for at least 20 months [8].

AGE contains many important water-soluble organosulfur compounds with potent antioxidant and free radical scavenging activities [8,9]. So far, AGE has been demonstrated to possess several physiological activities in experimental animals, including vasodilative and hypotensive activities, the induction of decrease in serum cholesterol levels, antimicrobial, antiallergic, anti-inflammatory, immunomodulatory, and antioxidant properties [8,9]. Recently, AGE has received particular attention because of studies that have reported that it is a highly efficient antioxidant and has free radical scavenging capacity [9,10].

The present study aimed to investigate the possible protective effect of AGE on some hematological parameters, as well as on the activity of antioxidant enzymes and lipid peroxidation in liver of rats treated with CP.

## 2. Materials and methods

### 2.1. Chemicals

CP was obtained in the form of commercial Egyptian Unistin Vial (Egyptian International medical Company (EIMC) United Pharmaceuticals, Cairo, Egypt). AGE (kyolic) was obtained from Wakunaga of America (Mission Viejo, CA). It was prepared by soaking sliced raw garlic (*Allium sativum*) in 15–20% aqueous ethanol for at least 10 months at room temperature. The extract was then filtered and concentrated under reduced pressure at low temperature. The content of water-soluble compounds was relatively high while that of oil-soluble compounds was low. The AGE used in this study contained 28.6% extracted solids (286 mg/ml), and S-allyl cysteine, the most abundant water-soluble compound in AGE, was present at 1.47 mg/ml.

### 2.2. Animals

Twenty-four adult male Wister albino rats (12–14 weeks of age) were obtained from the animal house, Faculty of Medicine, Zagazig University, Zagazig, Egypt. The rats were kept under appropriate conditions of temperature ($25 \pm 2\,^{\circ}C$), humidity (60–70%) and light (12 h dark/light cycle), free access of a commercial balanced diet and tap water ad libitum.

### 2.3. Experimental design

After one week of acclimatization, the animals were randomly divided into four equal groups in separate plastic cages, six rats each. Two groups (I and II) were used as control and received normal saline 0.5 ml i.p. and distilled water P.O. (group I) and AGE, 250 mg/kg orally (group II) for 21 days. Groups (III and IV) received single i.p. dose of CP (7.5 mg/kg) on day 16th, after successive administration of distilled water (0.5 ml, orally, group III) or AGE (250 mg/kg orally, group IV). The rats of each group were weighed on 1st, 4th, 7th, 10th, 13th, 16th, 19th and 22nd days.

### 2.4. Samples collection

On day 22th, (6 days after CP injection), the rats were anesthetized by ether inhalation. *Blood samples* were collected through a direct intracardiac puncture from each rat. Two blood samples from each rat were collected one sample was collected on EDTA (heparinized tubes) for determination of hematological parameters and the other was left to clot at 37 °C and centrifuged at 3000 rpm for 15 min. The serum (supernatant) was collected and stored at −20 °C for biochemical analysis.

*Tissue samples*: A vertical midline thoracic and abdominal incision was done to explore their viscera. Liver of each animal was excised, cleaned from their surrounding fat and connective tissue, washed with normal saline, blotted with filter paper, weighed and rinsed in ice-cold saline. Half of each liver was homogenized for biochemical analysis and the other half was processed for histological examination.

### 2.5. Hematological studies

The heparinized blood samples were analyzed for the number of red blood cells (RBCs), white blood cells (WBCs), and platelets, hemoglobin concentration (Hb%), hematocrit value (HTC), packed cells volume (PCV), mean corpuscle volume (MCV), mean corpuscle hemoglobin (MCH), mean corpuscle hemoglobin concentration (MCHC) and the differential count of polymorphs and lymphocytes according to standard methods using an Animal Blood Counter-ABC vet (Horiba ABX, France).

### 2.6. Biochemical assays

*Liver biomarkers assessment*: the levels of aspartate transaminase (AST) and alanine transaminase (ALT) enzymes were estimated in the sera of the blood samples using commercial kits (Roche Diagnostics, GmbH, D-68298, Mannheim, Germany) according to Reitman and Frankel [11]. Also, serum albumin was determined using commercial kit supplied by Diamond, RA50, Ireland and total serum bilirubin (TSB) was assayed according to the method of Schmidt and Eisenburg [12] as well.

*Lipid peroxidation and antioxidants assessment*: Half of each rat's liver was minced and homogenized in ice-cold 10% trichloroacetic acid phosphate buffer saline (0.05 M,